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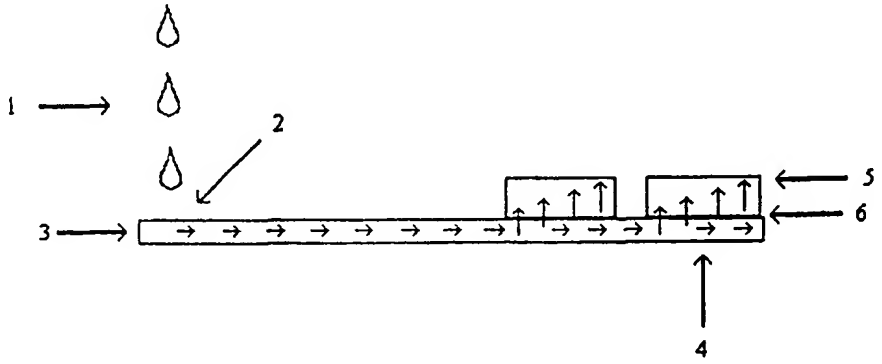
(54) Title: METHOD FOR DETECTION OF ANALYTES IN URINE USING LATERAL FLOW HYBRID DEVICE

(57) Abstract

This invention is in the field of toxicology and clinical chemistry. The invention relates to dry chemistry test devices which can be used in Point of Care testing in the detection of analytes, including analytes pertaining to adulteration of urine samples submitted for drugs of abuse testing as well as analytes for clinical chemistry parameters. The test device prevents cross contamination and increases accuracy and precision.



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METHOD FOR DETECTION OF ANALYTES IN URINE USING LATERAL FLOW HYBRID DEVICE

BACKGROUND OF THE INVENTION

1. Related Applications

This application is a continuation-in-part of application serial number 09/290,631 filed April 8, 1999 entitled "Method for Detection of Glutaraldehyde and Other Analytes in Urine Using Dry Chemistry Test Pads and Lateral Flow" and a continuation-in-part of application serial number 09/323,922, filed June 2, 1999 entitled "Method for Transporting Fluids Through a Lateral Flow Medium to a Dry Chemistry Test Pad".

2. Field of the Invention

The field of this invention is a test device which may be used for adulteration testing, that is, to determine whether body fluid samples submitted for drug testing have been tampered with or "adulterated". The invention may also be used in the field of in vitro diagnostics chemistry testing. The test device of the present invention makes use of lateral flow mechanics and dry chemistry techniques to present surprising and unexpected multiple advantages over the prior art. This device can be utilized in "Point of Care" ("POC") products.

3. Brief Description of the Prior Art

As the use of illicit drugs in this country has increased, public concern over the problems associated with its effects has grown into a major concern. This concern has led to workplace drug testing in order to identify, treat, and remove active drug users from the workforce. This trend started in the military, and spread rapidly to law enforcement and any "safety-sensitive" private sector jobs such as airline pilots, truck drivers, and active crew members of public transportation. These initial strides into drug testing in the workplace revealed the obtrusive incursion of drug use and abuse in the daily lives of a significant portion of Americans. Further research indicated the staggering costs to public and private industry in terms of lost productivity, increased health care costs, and human suffering and death due to this scourge of drug abuse. As a result, drug testing has rapidly spread to all areas of the public and private sector. The vast majority of workplace drug testing has taken the form of urine testing, because of ease of collection, low cost, and

effective indication of recent drug use. Other forms of testing include analysis of blood, saliva, sweat, and hair.

Because the effects of a positive test on the individual can be significant, and traumatic, the analysis procedures must guarantee accuracy with the emphasis on zero false positive results. On the other hand, all efforts must be made to detect all drug users in order to insure the success of this policy. These two requirements dictate a policy of close and vigorous scrutiny of the collection, testing, and reporting procedures. Juxtaposed to these closely monitored procedures is the deep and abiding desire of illicit drug users to avoid detection in order to keep their use secret, and to keep their jobs. Thus driven by these key desires, the ingenuity of a few in the drug abuse subculture has led to a plethora of ways to defeat the workplace drug testing procedures. These "adulteration " methods all conspire to produce the same desired effect: a false negative result which will protect the drug user's secret.

Adulteration techniques can be divided into two distinct types. The first utilizes an "in vivo" technique in which the user consumes the adulterant. The second technique utilizes an "in vitro" method in which the abuser adds the adulterant directly to the urine specimen submitted for testing.

The drug testing procedure involves two distinct parts. The initial segment is a panel of screening tests for the individual drugs. If a positive result is obtained in any of these initial tests, then a confirmation assay is performed for each drug that screened positive. Most adulteration techniques are aimed at the screening process, because of the inherent fragile nature of these inexpensive assays which adapt well to rapid, automated analysis techniques. All screening tests utilize antibody/antigen reactions quantified via an enzyme indicator. On the other hand, confirmation assays are labor and time intensive, highly accurate, expensive, and more difficult to adulterate. In addition, the positive screen has already raised a red flag, thereby drawing attention to the sample. The confirmation analysis utilizes GC-MS (gas chromatography mass spectrometry) testing which is considered the "gold standard" for drug assays scientifically and legally.

The "in vivo" methods function in one of three ways. These include dilution of the analyte of interest to a level below that required for a positive result, decreasing the time

required to eliminate the consumed drug, or consuming a compound that will interfere with the screening method. Dilution is effected by consuming a large volume of liquid together with a diuretic to speed elimination of urine, and a B vitamin to add yellow color to the urine sample. Some commercial in viva dilution products or "flushes" are sold under the following names: Carbo Clean, Test Pure, Kleen Test, Quick Flush, Naturally Klean, Test Free, UA Flush, Zydot's Special Blend, Daily Pure, Vale's Quick Clean, Test'n, and UR'n Kleen. Decreasing the elimination time will often enable the weekend drug user to avoid testing positive on a Monday morning drug test. This is accomplished by consuming acidic liquids (e.g. acidic fruit juices or ammonium chloride) to speed up elimination of basic drugs, or consuming basic liquids to speed up elimination of acidic drugs. Examples of an internally ingested substance which will disrupt the screening test procedure include aspirin and mefenamic acid, a prescription analgesic pain killer.

In vitro methods utilize literally hundreds of products and compounds that will adversely affect either the screening or confirmation process. Products affecting the screening process include many household products (i.e. all types of cleaners including hand, clothes and dishwashing detergents and soaps, table salt, bleach (sodium hypochlorite), fingernail polish remover, vinegar, Drano, liquid plumber, sodium bicarbonate, Visine, fingernail polish, swimming pool cleaning chemicals and acid), or specialty products sold commercially as adulterants (i.e. Urine Luck, Purafyzit, Urine Sured, and THC Free are acid-based products, UrinAid and Clear Choice are glutaraldehyde containing products, Amber-13 contains sulfides, Mary Jane Super Clean 13 is a soap, Stealth, and Toxicleen). Commercial products aimed at interfering with the confirmation process include nitrite containing products Klear and Whizzies.

Substitution, or using a clean urine sample supplied by a third party, can be either in vivo or in vitro adulteration. In its simplest form, participants hide a clean urine in their clothing and put it into the specimen collection container (in vitro). Individuals requiring more stealth including those giving observed collections (military and corrections primarily) may substitute via the in viva technique which requires putting the clean urine into the subject's bladder using a catheter.

Illicit drug users have learned to falsify urine screening tests by in vitro adulteration of urine samples by the addition of several readily available agents, including household products (soap, bleach, etc.), hydrogen peroxide, and commercially available adulteration products, such as "UrinAid" (glutaraldehyde).

The vast majority of urine collections are not observed due to privacy issues. Collection facilities try to prevent in vitro adulteration or substitution by recording the temperature of the sample as soon as it is collected. It must fall inside the very narrow range of 90.5 to 99.8 degrees Fahrenheit. They also may require subjects to leave excessive clothing out of the collection room, and provide no hot water which prevents dilution of the sample with water. Obviously, however, it is very easy to secret small quantities of adulterating substances into the collection room. As little as a pinch of salt or a drop or two of glutaraldehyde or acid will affect most test screens. Because the effective amounts of most adulterants are very small, even observed collection as required by the military and criminal justice system can be defeated using the in vitro technique.

On the other hand, collection facilities currently have no weapons to detect in vivo adulterants, because they are consumed by the drug user several hours or days prior to collection of the sample. Currently only certain forms of adulteration as already mentioned can be detected in the laboratory.

All screening assays can be adulterated. These assays fall into three types of methods: florescent polarization immunoassay (FPIA), radioimmunoassay (RIA), and enzyme immunoassay (EIA, CEDIA, KIMS: Antibody/agglutination). Toxicology and clinical chemistry literature includes numerous studies on the effects of adulterants on the screening technologies and the recommendation to test for their presence in urine samples. References include Mikkelsen and Ash, "Adulterants Causing False Negatives In Illicit Drug Testing", Clin. Chem. 34/11, 2333-2336 (1988); and Warner, "Interference of Common Household Chemicals In Immunoassay Methods For Drugs Of Abuse", Clin. Chem., 35/4, 648-651 (1989).

Accordingly, a need exists for providing an easy end convenient manner by which to make a determination of the presence of adulterants in urine samples which are being tested for drugs of abuse and, similarly, to test for analytes in urine or other samples (not

necessarily for Drugs of Abuse ("DAU"). A further need exists for a convenient manner by which such determinations may be made by using rapid analysis manual techniques (such as a dipstick or lateral flow device).

One embodiment of the present invention is test strips (i.e. dry chemistry dipsticks, or on-site test modules utilizing thin layer chromatography in a lateral flow format, or other technology similar to the test strip) and liquid chemistry reagents for use in the detection of various adulterants or parameters which indicate adulteration of immunoassays designed to detect DAU in aqueous fluids, including urine, saliva, serum, blood, sweat extracts, and liquid homogenates of hair. Enzyme and antigen/antibody reaction kinetics are related to the rate of change in analytical, biological systems. The variables that affect this rate of change include concentration of reactants and product, temperature, pH, ionic strength, buffer strength, and other parameters. Many commercial and household adulterants dramatically affect the parameters noted above.

Currently, all adulteration detection assays actually analyze the test sample itself for physical or chemical abnormalities. For instance, most of the adulterants noted above can be identified by determining the pH, or specific gravity of the sample. Assays for these parameters or characteristics will detect the presence of acids, bases, table salt, and high ionic strength soaps and detergents. Prior to the present invention, there were no dry chemistry (dipstick or lateral flow technology) commercial tests available for glutaraldehyde-based adulterants (i.e. UrinAid and Clear Choice's Instant Clean ADD-IT-ive).

The device of the present invention clearly indicates whether the adulterant is present in the sample matrix and thus prevents a false positive or false negative result if only the drug test were relied upon. Currently, the adulteration underground network produces two to five new methods to fool the drug tests each year. As a result drug testing facilities are forced to maintain a constant vigil for any unusual results. It is well known that adulteration of samples submitted for DAU testing cost the drug testing laboratories, employers, taxpayers and everyone involved in the drug screening process countless millions of dollars every year.

GC-MS, a confirmation, assay, is performed to verify the urines that screen positive for drugs of abuse. The GC-MS analysis costs 100 times as much as the initial screen ($100 vs $1). Every additional unnecessary GC-MS performed drives up the overall cost of drug testing. Eliminating these additional, unnecessary assays will save millions of dollars per year. False positive drug screens also strongly impact on-site testing. In most situations utilizing POC_2 on-site tests (on site devices such as dipstick or lateral flow devices require no instrumentation, making these devices ideal for collection and on site facilities) the employee is screened upon arrival for work. If a positive is obtained using the on-site test, a second sample is forwarded to the lab for GC-MS confirmation and the employee is suspended from work or reassigned to other duties until the results of the test are known. Therefore, it is of vital importance that the employer and laboratory know if the sample has had an adulterant added, to save time, money, and possibly lives.

Thus, a need exists in the field of workplace drug testing for rapid, economical, and effective adulteration analysis of samples submitted for testing, particularly for POC testing.

This invention provides a previously unavailable dry chemistry method for determining adulteration of a test sample by measuring the presence of glutaraldehyde as required currently by the Department of Transportation, Nuclear Regulatory Commission, College of American Pathologist's Forensic Urine Drug Testing program, and the U.S. Department of Health and Human Service's SAMSHA program on all urine assayed for drugs of abuse under their protocols.

The ease of use and rapid results obtained by the present invention illustrates the unique utility of this testing technique. In addition, very little technical expertise is required to perform this type of assay (no instrumentation is required). Furthermore, the early detection of adulteration (prior to DAU screening) facilitates faster replacement with a fresh, untainted specimen from the participant, yielding more accurate information. The present invention thus presents the opportunity for significant economic savings.

Current POC test devices are utilized in a wide variety of ways. For instance, in the drugs of abuse testing field, POC devices utilize a lateral flow format to detect drugs of abuse. That is to say, that a few drops of urine specimen is added to one end of lateral flow

device material. The urine then migrates to the opposite end of the material. During this process the urine will interact with chemicals or reactants in that are impregnated into the paper and yield a detectable response. In the clinical chemistry field, urinalysis dipsticks utilize a dry chemistry urine dipstick. Dipsticks used in this manner typically have a plastic backing 5 mm wide by 70 mm long with several small pads 5 mm by 5mm (generally made of chemically impregnated filter paper) laminated onto the plastic backing with an adhesive. These strips are then dipped into a container typically filled with urine. The strips are removed and the reaction occurring on the chemically impregnated test pads is visually observed for a specific color reaction. That color is then compared to a color chart that has a range of colors specific to the test pad which correspond to a particular concentration of the analyte of interest. One of many drawbacks of this type of method is that the urine which is left on the dipstick mixes with a chemically impregnated test pad and then crosses over to another test pad. In the devices of the prior art, this cross over contamination or cross-contamination could interfere with the chemical reaction of the second test pad and cause erroneous results. The dipstick of the prior art, by being dipped into the urine sample, could alter the integrity of the urine by contamination of the urine with the chemically impregnated test pads. This contamination could have legal implications in DOA testing where additional testing on the sample may be required.

POC devices are being used in an ever increasing rate as an inexpensive alternative to physician and hospital visits. Many POC devices are used once and discarded. It is an object of the present invention to improve accuracy, precision, prevent cross-contamination or carry over, and reduce cost to the end user. The device and method of the present invention allow for the detection of adulterants and other analytes effectively with a single assay using therefore according the user an easy and convenient manner by which to make a determination of the presence or absence of the analyte in samples. Further, the unique device of the present invention also eliminates interference from cross over of reagents or fluid from one test pad to another, a problem experienced with prior art dipsticks. The delivery of a known amount of fluid to the reaction pad improves precision, accuracy, and sensitivity of the test device. Applicant's invention also fills the need for a rapid, manual determination of adulteration.

SUMMARY OF THE INVENTION

The present invention relates to test devices for detecting the presence of an adulterant or other analyte in a liquid test sample such as urine, the device manufacture, and use. The invention may also be used to detect analytes typically associated with clinical chemistry. The present invention provides dry chemistry test strips (i.e. dipsticks, or dry chemistry and lateral flow [thin layer chromatography] test means) for use in the detection of adulteration of human, biological samples (e.g. urine, blood, serum, saliva, sweat extracts, and hair homogenates) to be tested for DAUs via immunoassays. This invention achieves this goal by measuring the absence or presence of adulterant or other analyte, or by testing for parameters which would indicate the presence of an adulterant in a test sample. One embodiment of the invention is a unique device which eliminates cross-contamination between reactant areas such as test pads on dipsticks by the use of both dipstick test pad and lateral flow device technology.

The present invention encompasses a method that can utilize several different techniques. The techniques employ a manual method using dry chemistry dipsticks and a method of combining dry chemistry dipstick reactant areas (test pads) with lateral flow thin layer chromatography. The present invention uses a novel method of lateral flow in combination with the test pad. The lateral flow and dry chemistry dipstick hybrid device is a rapid test that uses absorbent medium such as paper which has been impregnated with the chemical formulation (the test pad) that will detect various analytes from bodily fluid samples, such as urine, placed on top of a lateral flow medium.

In a lateral flow device, the reaction takes place within the lateral flow material (which can also be used as support material). The reaction takes place on the dry chemical test paper, not the plastic backing or support material. In the lateral flow format, a few drops of urine specimen can be added to one end of lateral flow device material (usually a paper matrix of some type, nitrocellulose, for instance). The urine then migrates to the opposite end of the material. During this process the urine will interact with chemicals or reactants that are impregnated in the paper matrix to yield a detectable response. Thus, the reaction takes place inside of the lateral flow material. One advantage of the lateral flow technology is that the lateral flow material will limit the maximum amount of sample

which will be used. The amount of sample used will be the same as long as the material is the same. This advantage allows for improved precision and accuracy.

Dry chemistry dipsticks use a chemically impregnated test pad. The dipsticks typically have a plastic backing 5 mm wide by 70 mm long with several small pads (each representing a different test) 5 mm by 5 mm (generally made of chemically impregnated filter paper) laminated onto the plastic backing with an adhesive. These strips are then dipped into a container typically filled with urine. The strips are removed and the reactions upon the chemically impregnated test pads are visually observed for specific color reactions. The test pads are then compared to a color chart that has a range of colors specific to the test pads, which correspond to a particular concentration of the analyte of interest. The reaction takes place in the test pad not on the backing or support material. The dipstick technology does not quantitate the volume of liquid sample used.

In the present invention, a filter paper (or other suitable material capable of absorbing chemicals), after impregnation with chemicals (the test pad) that will detect the parameter or analyte of interest, is placed on a lateral flow medium, such as nitrocellulose paper, glass fiber paper, or other suitable wicking material. The wicking material delivers the test sample to the impregnated paper. The device thus has both dipstick test pads (the filter paper) and lateral flow characteristics and will be referred to herein as the Lateral Flow Hybrid ("LFD Hybrid") device. The LFD Hybrid device works by dipping one end of the device into a sample of urine, dropping a few drops of urine onto one end of the device or otherwise contacting the sample with the device. The fluid then migrates up (along) the paper (or absorbent material) to the reactive sites where the filter paper is in fluid contact with the nitrocellulose lateral flow material. The filter paper then absorbs the fluid from the lateral flow paper and a detectable chemical reaction takes place.

The LFD Hybrid device has a quantitative to qualitative assay range. The results are evaluated via one of two categories: negative and positive or given in quantitative ranges. For example, a pH result could be determined from a pH range of 3.0 to 1 1.0 pH units in increments of 0.1 pH units.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 and 1B are side views of the preferred embodiment of the test device.

FIG. 1A is a top plan view of the preferred embodiment of the test device.

FIG. 2 is a side view that depicts the lateral flow material resting on the top edge of the test pad of the test device.

FIG. 3 is a side view of the test device with the lateral flow material in vertical contact with the test pad.

DETAILED DESCRIPTION OF THE INVENTION

An embodiment of the present invention is a device in the form of a dry chemistry dipstick or LFD Hybrid for the detection of adulterants in sample matrices consisting of urine or other biological specimens. The assay device includes an adulteration reagent comprising buffer(s) and color indicator(s). Buffering of the reactants is critical to the adulteration reagent, because pH plays a vital role in the reaction kinetics. In the case of the dipstick and the LFD Hybrid, adulteration reagent components are impregnated on a test strip pad composed of solid, absorbent carrier(s) matrix, usually known as test pads. In the dipstick, these test pads are typically affixed to a solid support (usually plastic). The dipstick is then submerged in the liquid test sample, removed, and a measurable (i.e. visible) response is observed. In the LFD Hybrid, the dry chemistry test pad is chemically impregnated in the manner used with the dipstick. The test pad is then placed in fluid (direct) contact with lateral flow paper (such as nitrocellulose). This device is then exposed to a sample fluid such as urine. The urine migrates to the location of the test pad, saturates the test pad, and the reaction takes place.

The adulteration reagent used in the dipstick and the LFD Hybrid comprise one or more compositions which produce a detectable manifestation of the presence or absence of a particular chemical component. The chemical component being detected is that adulterant (e.g., glutaraldehyde, chromate, bleach such as household bleach, oxidant, nitrite) or an indication of adulteration (e.g., altered pH, specific gravity, creatinine) or a chemistry parameter (e.g. calcium, glucose, urobilinogen, bilirubin, protein, ketone, leucocyte esterase) in the sample. The response can be in the form of the appearance or disappearance of a color, or the changing of one color to another. Said measurable

response may also be evidenced by a change in the amount of light reflected or absorbed during the reaction of interest. Those skilled in the art are familiar with methods of detectable responses.

One feature of the present invention are newly discovered indicators for glutaraldehyde in urine: sodium nitroprusside, ferric chloride, salicylates, phenol, antipyrine, and sodium nitroprusside analogs.

Buffers to maintain the appropriate pH will be required. Suitable buffers may include any of the following (referred to here by their commonly used acronyms): citrate, borate, borax, sodium tetraborate decahydrate, sodium perchlorate, sodium chlorate, sodium carbonate, tris base, tris acid, MES, BIS-TRIS, ADA, ACES, PIPES, MOPSO, BIS-TRIS PROPANE, BES, MOPS, TES, HEPES, DIPSO, MOBS, TAPSO, TRIZMA, HEPPSO, POPSO, TEA, EPPS, TRICINE, GLY-GLY, BICINE, HEPBS, TAPS, AMPD, TABS, AMPSO, CHES, CAPSO, AMP, CAPS, CABS, hydrochloric acid, phosphoric acid, lactic acid, potassium hydrogen tartrate, potassium hydrogen phthalate, calcium hydroxide, phosphate, bicarbonate, sodium hydroxide, potassium hydroxide, oxalate or succinate. Other buffers with an effective pK and pH range, and capacity suitable for maintaining the sample-reagent mixture within the required parameters of the assay's reaction mechanism may be added to the above group. Alkaline buffers are preferred for the glutaraldehyde assay. A large number of the chemical names for the common named compounds above may be found in the 1999 SIGMA® catalog pages 1910 through 1917.

Manufacture of the dry chemistry dipsticks and the test pads used on the LFD Hybrid may require the addition of thickeners as taught in the art. Some compounds commonly used for this purpose include: polyvinylpyrrolidone, algin, carragenin, casein, albumin, methyl cellulose, and gelatin. The typical range of concentration for these thickeners is about 0.5 to 5.0 g. per 100 ml. Wetting agents or surfactants are also typically used in dry chemistry. For dry chemistry applications, wetting agents aid in even distribution of the chemicals and promote even color development. Acceptable wetting agents can be hydrophilic polymers, or cationic, anionic, amphoteric, or nonionic species. Some commonly used wetting agents include sodium dodecyl-benzene sulphonate, sodium lauryl sulphate, benzalkonium chloride, N-lauroylsarcosine sodium salt, Brij-35, Tween 20,

Triton X-100, dioctyl sodium sulphosuccinate, and polyethylene glycol 6000. Wetting agents can be added to dipstick impregnation solutions in amounts of 0.5% to 5.0%, and 0.1% to 1.0% in liquid reagents.

Color enhancers may be required or used such as sucrose, lactose, glucose or other compounds. Color enhancement can be defined as intensification and /or alteration in some manner the color that is produced by the reaction to improve the measurement of the detectable response.

The dry chemistry test strips/test pads for the present invention can utilize any form of absorbent, solid phase carrier including paper, filter paper, filter membrane to include polyvinyl, polyvinylidene fluoride, polyester polyethersulfone, polytetrafluoroethylene, cellulose acetate, cellulose nitrate, cellulose acetate, nylon, cellulose esters, acrylic copolymer, polypropylene, nitrocellulose, mixed esters of cellulose nitrate and cellulose acetate, polycarbonate, teflon, glass fiber paper, crepe filter paper, wood pulp paper, cotton filter paper, ashless filter pulp, cotton linter filter paper, cellulose, synthetic resin, fleeces, or other suitable absorbent material in conjunction with liquid solutions of reagent compositions in volatile solvents. The test strip/test pad material should be an absorbent (wicking) material that permits migration of sample up the solid absorbent test pad and allows analytes and reactants to react. The impregnation may be in one or more steps. Each impregnation may contain one or more of the chemical compounds making up the assay reagent composition; the exact procedure is dictated by the inter-reactivity of the assay constituents and the order in which they may have to react with the analyte of interest.

The LFD Hybrid lateral flow material can utilize any form of absorbent, solid phase carrier that is capable of transporting a fluid. These can include filter paper, cellulose or synthetic resins. More specifically, the lateral flow material can include cellulose, cellulose acetate, nitrocellulose, mixed ester, teflon, polyvinylidene difluoride (PVDF), polytetrafluoroethylene (PTFE), polysulfone, cotton linter, non-woven rayon, glass fiber, nylon, ion exchange or other suitable membranes or solid support. Preferably, the solid phase carrier that has the capabilities of transporting a fluid from one site to another across

a distance of at least 5 to 10 mm. In some cases, the lateral flow material may also serve as a support material.

After impregnation, the solid phase carrier of the dipstick is dried, cut into strips, glued to a support structure (usually a flexible, flat, plastic stick) as part of a "sandwich" composed of a handle, a test pad, and a synthetic resin film and/or a fine-mesh material in the manner described in German Pat. No, 2,118,455, incorporated herein by reference. In the preferred embodiment, the impregnated test pad is attached to or put into contact with lateral flow material. In another embodiment, the present invention may be combined with the water-stable film as taught in U.S. Pat. No. 3,530,957, incorporated herein by reference, to produce a dipstick in which the excess sample fluid can be wiped off in order to improve the accuracy and precision of the results.

The LFD Hybrid comprises some or all of the following: test pad (usually filter paper) impregnated with buffers, and reaction components that can include indicators, surfactants or other ingredients needed for the test pad to be reactive to an analyte of interest. The test pad is then placed in fluid contact with the lateral flow material. This device is then exposed to a fluid. The fluid then migrates to the location of the test pad, saturates the test pad, and the reaction takes place.

Referring now to FIG. 1, the liquid sample 1 is introduced through pipetting, dropping, dipping, contacting or exposing the liquid sample 1 in some manner to the sample introduction area 2 of the lateral flow material 3. The sample 1 then migrates (as illustrated by the arrows) from the sample introduction area 2 to opposite end of the lateral flow material 4. While the sample 1 is flowing from the sample introduction area 2 to the opposite end 4 of the lateral flow material 3, the chemically impregnated dipstick test pad 5 (which is in fluid contact with the lateral flow material 3) will become saturated with the sample 1. The chemical reaction will occur between the test pad 5 and the sample 1 producing a detectable response. FIG. 2 depicts the lateral flow material 4 as placed onto the top edge of the chemically impregnated dipstick test pad 5. Thus, when the fluid sample 1 reaches the edge of the dipstick test pad 5, the test pad 5 becomes saturated with the sample 1 in a similar manner as FIG. 1 and the chemical reaction takes place and a detectable response occurs. In FIG. 3, the lateral flow material 4 is placed next to the

chemically impregnated dipstick test pad 5 (but, still in direct (fluid) contact with the lateral flow material 3). FIG. 1B illustrates multiple test pads 5 which are in contact with the lateral material 4. As the fluid migrates from one pad to the next, no cross over from one test pad 5 to the next occurs, thus, preventing cross contamination. The pads allow for a specific and constant amount of fluid to reach each pad, enhancing precision, accuracy, and specificity.

The following examples are provided to further illustrate the present invention and to further exemplify preferred embodiments. As such, they are intended as merely illustrative, and are not to be construed as limiting the scope of the claims appended hereto. The concentrations of the components in the following examples can be varied to suit the dipstick device format, dependent upon paper type, or use of semi-permeable membrane or other suitable material. Further, the concentrations of the constituents can be varied to suit the LFD Hybrid device format, e.g. dependent upon paper type, and inclusion of semi-permeable membranes or other innovations utilized in dry chemistry technology. The color charts referred to in the following examples can be made in-house using standard techniques available to those skilled in the art. Alternative sources are available.

EXAMPLE 1

This example illustrates a dry chemistry dipstick (test strip) with a solid carrier for the adulteration detection of glutaraldehyde in samples submitted for drugs of abuse analysis and its use.

Filter paper was successively impregnated with the following solutions and dried at 25 degrees C:

Solution 1:

Disodium phosphate (sodium phosphate dibasic) 200.0 g

Sodium nitroprusside 20.0 g

distilled water added to 1 L total volume of solution

pH of the solution should be between 7.5 and 11.5

The dipstick comprised a paper carrier or solid matrix incorporated with the composition of Solution 1. In this example, to produce the test means, a piece of

Schleicher & Schuell (Keene, NH 03431) 593 grade filter paper having approximate dimensions of 1 inch by 3 inches was impregnated with Solution 1 by immersing the paper into Solution 1. The paper was then dried by using forced air not exceeding 25° C. The dried paper was then applied (laminated by pressing the paper and adhesive together) to one side of a piece of double-sided adhesive transfer tape commercially available from 3M Company (St. Paul, MN 55144). The laminate was then slit into sections measuring 3 inches by 0.2 inches. One section of the laminate was then attached, via the unused adhesive side to a polystyrene sheet measuring about 1.5 inches by 3 inches. The resulting laminate was slit parallel to its short dimension to form test devices comprising a 1.5 inch by 0.2 inch oblong polystyrene strip carrying a square of the impregnated papers at one end, and the other end serving as the handle. The dipstick worked as follows after contact with a urine sample: if no uniform dark gray to brown color developed, then no glutaraldehyde was present. Conversely, if any concentration of glutaraldehyde was present in the urine at a 0.1 % v/v or greater, a dark gray to brown color developed, thus confirming the presence of glutaraldehyde.

EXAMPLE 2

This example pertains to a method for manufacturing the LFD Hybrid for glutaraldehyde testing. This method could also be utilized for any general chemistry "test pad" or pads that are currently used or will be used in the art. The manufacturing process includes impregnating onto an absorbent, solid carrier (e.g. paper) called in this example, the "test pad", in the same manner as Example 1 with similar constituents. The test pad, once impregnated, is dried, then mounted onto a solid support (nitrocellulose membrane) that is capable of transporting (through lateral flow) liquid to the test pad from the point of application of a test sample. The device is dipped into a liquid or the liquid sample is placed on the device at the bottom or starting point for the assay. The liquid migrates from the starting application point to the opposite end of the nitrocellulose lateral flow paper, during which the test pad becomes saturated with the sample. The reaction takes place on the test pad and color develops. The developed color is then compared to a color chart with known concentrations of glutaraldehyde that has the appropriate colors relative to each specific concentration of glutaraldehyde. The results are then recorded. The test pad must

be an absorbent (wicking) material that permits migration of sample up the solid absorbent test pad and allows analyses and reactants to interact.

Absorbent material was successively impregnated with the following solutions and dried at 25 degrees C.:

Solution 1

0.05 M Borax

ferric chloride 20.0 g

distilled water added to 1 L total volume of solution

pH of the solution should be between 8.0 and 11.0

A paper carrier matrix (S&S, 593 grade filter paper) was impregnated with the compositions of Solution 1. The paper was then cut into test pads 5 mm by 5mm. The paper was then dried using forced air. The dried impregnated test pad was then placed at approximately 35 mm (in the middle) of a 5 mm wide by 70 mm long nitrocellulose membrane (S&S FastTrack™ NC) and made fluid contact with nitrocellulose lateral flow paper. The nitrocellulose membrane was capable of transporting a liquid by capillary action or wicking from one end of the LFD Hybrid device to the other in approximately 60 seconds. In this example, the LFD Hybrid had the dimensions of 5 mm wide by 70mm long and could be backed by or in contact with strips of glass fiber filter material (e.g. S&S 30 grade) to aid in controlling the wicking action. Another solid support material can be used for this purpose.

The sample was placed on the test device 5 mm from one end of the strip, and 35 mm from the site of where the test pad was placed in fluid contact with the strip. For simplicity, this example had the 5 mm by 5 mm impregnated test pad placed on top of the lateral flow paper and thus in fluid contact with the said paper. The test pad can be placed on top of the lateral flow paper making fluid contact with the lateral flow paper from the bottom side of the test pad, or the lateral flow paper can touch the paper from the side of the test pad and remain in fluid contact with the test pad. Or the lateral flow paper can rest on top of the edge of test pad or be attached and in fluid contact with the test pad in some other manner.

One of the surprising results in using a hybrid device made of lateral flow material and a dry chemistry test pad is the lack of cross-contamination from one pad to the next from excessive fluid. This cross-contamination is a problem in the prior art devices. Currently there are available many different types of dry chemistry test strips available, such as the Miles Laboratories, Inc. MULTISTIX® test strips. The Miles product and many others like it has multiple reagents test pads with different chemical reagents impregnated onto each pad on a single support membrane backing (usually plastic). Because of the relative proximity of these pads to each other on the same device it is easy for cross contamination to occur and thereby cause unreliable results. This is called "runover" (i.e. when a reagent from one pad runs over another adjacent test pad). The present invention eliminates runover. The dipstick with multiple test pads is dipped into the urine sample. Thus, reagent from one pad can run over the other adjacent test pad. The present invention avoids this problem. The multiple test pads are not dipped into the urine. Instead, the urine is placed at the end of the device and travels through the lateral flow material to the reactant test pad.

The LFD Hybrid was used as follows. A drop of urine (approximately 50 uL) was applied at the starting point or origin of the strip. The urine migrated to the opposite or terminal end of the strip. As the urine migrated across the lateral flow material (nitrocellulose) and came into contact with the test pad (filter paper), the urine saturated the pad and caused a chemical reaction between the impregnated chemicals and glutaraldehyde (if present) in the urine. A gray to brown color developed on the test pad indicating a positive (greater than 0.1% glutaraldehyde) for the presence of glutaraldehyde. The color was compared to a color chart showing the different colors from gray to brown depending upon the concentration of the glutaraldehyde, if greater than 0.1%. If the sample did not contain glutaraldehyde in a concentration in excess of 0.1%, no reaction occurred and no color developed, indicating a negative result. This negative result color was then compared to the color chart. The reaction on the test pad was rapid and thus the test result could be observed and determined immediately.

EXAMPLE 3

This example illustrates a LFD Hybrid for pH. Filter paper was successively impregnated with the following solutions and dried at 25 degrees C:

Solution I

Bromthymol red 1.0 g

methyl red 0.5 g

distilled water added to 1 L total volume of solution

No buffer necessary, would affect the pH indicator's ability to response to changes in pH.

A paper carrier matrix (S&S, 593 grade filter paper) was impregnated with the compositions of Solution 1. The device was then made as set forth in EXAMPLE 2.

The LFD Hybrid for pH worked in a manner similar to that of the device in EXAMPLE 2. As the urine migrated across the lateral flow material (nitrocellulose) and came into contact with the test pad (filter paper), the urine saturated the pad and caused a chemical reaction between the impregnated chemicals and the urine. This reaction was instantaneous. A color was produced that correlated to a particular pH. By using a color chart with known concentrations and their corresponding colors a result could be determined when the color chart is compared to the LFD Hybrid device for pH.

The LFD Hybrid device is capable of measuring pH in the range from pH 5.0 to pH 8.0 in increments of 1.0 pH units. This constitutes the normal pH range for human urine. If the sample has a pH of 6.0 a particular color will be produced by the reaction of the urine and the chemicals impregnated on the test pad. The color on the LFD Hybrid device will match a color for pH 6.0 on the referenced color chart. Similarly, if the sample has another pH, the color for that pH will be produced.

EXAMPLE 4

This example illustrates a preferred LFD Hybrid for Chromate. Absorbent material was successively impregnated with the following solutions and dried at 25 degrees C:

Solution 1:

1,5-diphenylcarbohydrazide 0.01 g/L

1.0 N HCl (hydrochloric acid) added to 1 L total volume of solution

pH of the solution should be acidic

A paper carrier matrix (S&S, 593 grade filter paper) was impregnated with the compositions of solution 1. The device is then made as set forth in EXAMPLE 2. A drop of urine (approximately 50 uL) was applied at the starting point or origin of the strip. The urine then migrated to the opposite or terminal end of the strip. As the urine migrated across the lateral flow material (nitrocellulose) and came into contact with the test pad (filter page paper), the urine saturated the pad and (if a positive sample) caused a chemical reaction between the impregnated chemicals and chromate in the urine. If the sample was positive, with a concentration of 10 mg/dL chromate or more, the following occurred. A reddish purple color developed on the test pad indicating a positive (greater than 10 mg/dL chromate) for the presence of high levels of chromate. This color was compared to a color chart showing the different colors from colorless (white background) to a dark purple depending upon the concentration of the chromate, if greater than 10 mg/dL. If the sample was negative, with a concentration of less than 10 mg/dL of chromate present, no reaction occurred and no color developed, indicating a negative result. This negative result color was compared to a color chart. The reaction on the test pad was rapid and the test results could be observed immediately.

EXAMPLE 5

This example illustrates a preferred LFD Hybrid for Creatinine. Absorbent material is successively impregnated with the following solutions and dried at 25 degrees C:

Solution 1

100 g 3,5-Dinitrobenzoic acid

224.65 g Potassium Hydroxide

75 g Sucrose (7.5%)

Dissolve the above in 1 liter of DI water

Solution 2

100 g 3,5-Dinitrobenzoic acid

Dissolve the solution in 1 liter of isopropanol

A paper carrier matrix (S&S, 593 grade filter paper) was impregnated with the compositions of solution 1. The paper was then dried and impregnated with solution 2 then dried again. The device was then made as set forth in EXAMPLE 2.

A drop of urine (approximately 50 uL) was applied at the starting point or origin of the strip. The urine then migrated to the opposite or terminal end of the strip. As the urine migrated across the lateral flow material (nitrocellulose) and came into contact with the test pad (filter page paper), the urine saturated the pad and (if positive) caused a chemical reaction between the impregnated chemicals and creatinine in the urine. If the sample contained creatinine with a concentration of 5 to 10 mg/dL creatinine or more a purple color developed on the test pad indicating a normal urine creatinine concentration (i.e. greater than 10 mg/dL creatinine) or greater. Normal urine creatinine concentration can vary from 20 mg/dL to 400 mg/dL creatinine. This purple color was compared to a color chart showing the different colors from colorless (white background) to a dark purple depending upon the concentration of the creatinine. If the sample was negative, with a concentration of less than 10 mg/dL of creatinine present, no reaction occurred and no color developed, indicating a negative result. This negative result color was compared to a color chart showing the different colors from no color developed (negative). The reaction on the test pad was rapid thus the test results could be observed immediately.

EXAMPLE 6

This example illustrates a LFD Hybrid device for household, off the grocery store shelf, like bleach. Absorbent material is successively impregnated with the following solutions and dried at 25 degrees C:

Solution 1

1.0 M Tris HCl

0.5 g 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) ABTS

dilute the above ingredients to 1 liter with distilled water and the pH of the solution should be acidic (preferably around 2)

A paper carrier matrix (S&S, 593 grade filter paper) was impregnated with the compositions of solution 1. The device was then made as set forth in EXAMPLE 2. A drop of urine (approximately 50 uL) was applied at the starting point or origin of the strip. The urine then migrated to the opposite or terminal end of the strip. As the urine migrated across the lateral flow material (nitrocellulose) and came into contact with the test pad (filter page paper), the urine saturated the pad and, if positive, caused a chemical reaction between the impregnated chemicals and bleach in the urine. If the sample was positive, with a concentration of 1.0 % Bleach (sodium hypochlorite; off the grocery store shelf bleach) or more, bluish green color developed on the test pad indicating a positive (greater than 10 mg/dL bleach) for the presence of high levels of bleach. This color was compared to a color chart showing the different colors from colorless (white background) to a dark blue depending upon the concentration of the bleach, if greater than 1.0 % bleach in solution. If the sample was negative, with a concentration of less than 1.0 % bleach present, no reaction occurred and no color developed, indicating a negative result. This negative result color was compared to a color chart showing the different colors. The reaction on the test pad was rapid, thus the test results could be observed immediately.

EXAMPLE 7

This example illustrates the LFD Hybrid for Oxidant. ("Oxidant" as used in this example and application refers to oxidant screening; the test is not specific for any one type of oxidant and can detect, e.g. oxidants such as chromate, bleach, nitrite, and hydrogen peroxide as well as other oxidants.) Absorbent material was successively impregnated with the following solutions and dried at 25 degrees C.:

Solution 1

ABTS 0.01 g/L

0.01 N Tris [hydroxymethyl]aminomethane hydrochloride

pH of the solution of 2.0

pH of the 1 liter solution should be acidic

A paper carrier matrix (S&S, 593 grade filter paper) was impregnated with the compositions of solution 1. The device was then made as set forth in EXAMPLE 2. A drop of urine (approximately 50 uL) was applied at the starting point or origin of the strip.

The urine then migrated to the opposite or terminal end of the strip. As the urine migrated across the lateral flow material (nitrocellulose) and came into contact with the test pad (filter paper), the urine saturated the pad and, if positive, caused a chemical reaction between the impregnated chemicals and oxidant(s) in the urine. If the sample was positive, with a concentration of 1.0 mg/dL Oxidants or more, an orange color developed on the test pad indicating a positive (greater than 1.0 mg/dL Oxidants) for the presence of high levels of oxidants. This color was compared to a color chart showing the different colors from colorless (white background) to a dark orange depending upon the concentration of the oxidant(s), if greater than 1.0 mg/dL. If the sample was negative, with a concentration of less than 1.0 mg/dL of Oxidants, no reaction occurred and no color developed, indicating a negative result. This negative result color was compared to a color chart showing the different colors. The reaction on the test pad was rapid thus the test results could be observed immediately.

EXAMPLE 8

This example illustrates a LFD Hybrid for Nitrite. Absorbent material was successively impregnated with the following solutions and dried at 25 degrees C:

Solution 1

13.00 g p-Arsanilic acid

3.00 g N-(1-Napthyl)Ethylenediamine

21.00 g Tartaric Acid

7.00 g Sodium Carbonate

dilute the above ingredients to 1 liter with distilled water

A paper carrier matrix (S&S, 593 grade filter paper) was impregnated with the compositions of solution 1. The device was then made as set forth in EXAMPLE 2. A drop of urine (approximately 50 uL) was applied at the starting point or origin of the strip. The urine then migrated to the opposite or terminal end of the strip. As the urine migrated across the lateral flow material (nitrocellulose) and came into contact with the test pad (filter page paper), the urine saturated the pad and, if positive, caused a chemical reaction between the impregnated chemicals and bleach in the urine. If the sample was positive, with a concentration of 0.1 mg/dL nitrite or more, a pink to dark salmon color developed

on the test pad indicating a positive (greater than 0.1 mg/dL nitrite) for the presence of high levels of nitrite. This color was compared to a color chart showing the different colors from colorless (white background) to a salmon depending upon the concentration of the nitrite, if greater than 0.1 mg/dL in solution. If the sample was negative, no reaction occurred and no color developed, indicating a negative result. This negative result color was compared to a color chart. The reaction on the test pad was rapid thus the test results could be observed immediately.

EXAMPLE 9

This example illustrates an LFD Hybrid device for Glutaraldehyde. Absorbent material was successively impregnated with the following solutions and dried at 25 degrees C:

Solution 1

21 g of Sodium Nitroprusside

221 g Sodium Phosphate, dibasic

70 g Glycine

dilute the above ingredients to 1 liter with distilled water

A paper carrier matrix (S&S, 593 grade filter paper) was impregnated with the compositions of solution 1. The device was then made as set forth in EXAMPLE 2. A drop of urine (approximately 50 uL) was applied at the starting point or origin of the strip. The urine then migrated to the opposite or terminal end of the strip. As the urine migrated across the lateral flow material (nitrocellulose) and came into contact with the test pad (filter page paper), the urine saturated the pad and, if positive, caused a chemical reaction between the impregnated chemicals and glutaraldehyde in the urine. If the sample was positive, with a concentration of 10 mmol glutaraldehyde or more, a purple to gray slate color developed on the test pad indicating a positive (greater than 10 mmol of Glutaraldehyde) for the presence of high levels of glutaraldehyde. This color was compared to a color chart showing the different colors from colorless (white background) to a slate gray depending upon the concentration of the glutaraldehyde, if greater than 10 mmol in solution. If the sample was negative, with a concentration of less than 10 mmol Glutaraldehyde present, no reaction occurred and no color developed, indicating a negative

result. This negative result color was compared to a color chart. The reaction on the test pad was rapid, thus the test results could be observed immediately.

EXAMPLE 10

This example illustrates a LFD Hybrid device for Calcium. Absorbent material was successively impregnated with the following solutions and dried at 25 degrees C:

Solution 1

21 g of Arsenio III

1.0 M Tris HCl

dilute the above ingredients to 1 liter with distilled water

the pH of the solution needs to be acidic

A paper carrier matrix (S&S, 593 grade filter paper) was impregnated with the compositions of solution 1. The device was then made as set forth in EXAMPLE 2. A drop of urine (approximately 50 uL) was applied at the starting point or origin of the strip. The urine then migrated to the opposite or terminal end of the strip. As the urine migrated across the lateral flow material (nitrocellulose) and came into contact with the test pad (filter page paper), the urine saturated the pad and, if positive, caused a chemical reaction between the impregnated chemicals and calcium in the urine. If the sample was positive, with a concentration of 5 mg/dL urinary calcium or more, a purple color developed on the test pad indicating a positive (greater than 5 mg/dL calcium). This color was compared to a color chart showing the different colors from colorless (white background) to a purple depending upon the concentration of the calcium, if greater than 5 mg/dL. If the sample was negative, with a concentration of less than 5 mg/dL calcium present, no reaction occurred and no color developed, indicating a negative result. This negative result color was compared to a color chart. The reaction on the test pad was rapid thus the test results could be observed immediately.

EXAMPLE 11

This example illustrates a LFD Hybrid device for the detection of pH concentration in urine. Absorbent material was successively impregnated with the following solutions and dried at 25 degrees C:

Solution 1

0.5 g Bromcresol Green Sodium Salt

0.5 g Thymol Blue Sodium Salt

Add quantity of distilled water sufficient to bring to 1 L total volume of solution

Adjust the pH of the solution to 6.0 with 0.5 N NaOH or 0.5 N HCl solutions.

A paper carrier matrix (S&S, 593 grade filter paper) was impregnated with the compositions of solution 1. The paper can be impregnated by dipping the paper into Solution 1, or spraying the paper with Solution 1, or by some other suitable means. The paper was then cut into test pads 5 mm by 5mm in size. The paper was then dried using forced air. The 5 by 5 mm dried impregnated test pad was then placed onto a 5 mm wide by 70 mm long strip of nitrocellulose membrane (S&S FastTrack™ NC) at one end, so that the 5 by 5 mm test pad covered the last 5 mm of the 70 mm long strip and made fluid contact with nitrocellulose lateral flow paper. The nitrocellulose membrane was capable of transporting a liquid by capillary action or wicking from one end of the lateral flow device to the other in approximately 60 seconds. Therefore, the LFD Hybrid had the dimensions of 5 mm wide by 70mm and could be backed by or in contact with strips of glass fiber filter material (e.g. S&S 30 grade) to aid in controlling the wicking or capillary action. Other solid support material could also be used, such as paper, plastic, or vinyl or other suitable material to back the lateral flow material and provide support if needed.

The LFD Hybrid had a starting point or origin at which the sample was placed on the test device being 5 mm from one end of the strip, and approximately 60 mm from the site of where the test pad was placed in fluid contact with the strip. In this example, the 5 mm by 5 mm impregnated test pad was placed on top of the lateral flow paper and thus was in fluid contact with the said paper as shown in FIG. 1.

The starting point or origin at which the sample was placed on the test device was 5 mm from one end of the strip, and was approximately 60 mm from site where the chemically impregnated test pad was in fluid contact with the lateral flow paper as shown in FIG. 1. The test pad could be placed on top of the lateral flow paper making fluid contact with the lateral flow paper from the bottom side of the test pad FIG.1, or the lateral flow paper could touch the paper from the side of the test pad and remain in fluid contact

with the test pad as shown in FIG. 3., or the lateral flow paper could rest on top of the edge of test pad as shown in FIG. 2, or be attached and in fluid contact with the test pad in some other manner.

The results were interpreted as follows. A drop of urine (approximately 50 uL) was applied to the starting point or origin of the strip. The urine then migrated to the opposite or terminal end of the strip. As the urine migrated across the lateral flow material and came into contact with the test pad, the urine saturated the pad and caused a chemical reaction between the impregnated chemicals in the test pad and pH (buffering) ions in the urine. A color developed on the test pad indicating a specific pH. For this example, a green color will appear that correlated to a pH of 6.0. This green color was compared to a color chart showing the different colors from red pH 3.0 to a dark blue pH of 10.0 or greater. In this case, the color reaction of the test device produced a green color which indicated a pH of 6.0 when compared to the standard color chart which showed a green color for pH 6.0. The reaction on the test pad was rapid, thus the test results could be observed immediately.

If for example the sample had a pH of 3.0 then the color would be red since red was the color for pH 3.0 on the standard color chart.

EXAMPLE 12

This example illustrates an LFD Hybrid for the detection of glucose concentration in urine. Absorbent material was successively impregnated with the following solutions and dried at 25 degrees C:

Solution 1

4.0 g glucose oxidase

1.0 % urea peroxide

5.0 mL o-tolidine

1.0 N Phosphate buffer

Add a quantity of distilled water to provide 1 L total volume of solution

Adjust the pH of the solution to 7.0 with 0.5 N NaOH or 0.5 N HCl solutions.

A paper carrier matrix (S&S, 593 grade filter paper) was impregnated with the compositions of solution 1. The device was then made as set forth in EXAMPLE 11.

A drop of urine (approximately 50 uL) was applied to the starting point or origin of the strip. The urine then migrated to the opposite or terminal end of the strip. As the urine migrated across the lateral flow material (nitrocellulose) and came into contact with the test pad (filter paper), the urine saturated the pad and if positive, caused a chemical reaction between the impregnated chemicals in the test pad sensitive to the presence of glucose in the urine. A color developed on the test pad indicating a specific concentration of glucose. For this example, a blue color appeared that correlated to a glucose concentration of 50 mg/dL. The blue color was compared to a color chart showing the different colors from yellow (no glucose present) to dark blue, a glucose concentration of 500 mg/dL or greater. In this case, the color reaction of the test device produced a blue color which indicated a glucose concentration of 50 mg/dL when compared to the standard color chart which shows a blue color for 50 mg/dL of glucose. The reaction on the test pad was rapid and the test results could be observed between 15 and 30 seconds.

If for example the sample had no glucose present, the color would be yellow since yellow represents the color for no glucose present.

EXAMPLE 13

This example illustrates a LFD Hybrid containing two test pads on the same device as illustrated by FIG. 1B. The LFD Hybrid as shown in FIG. 1B specifically prevents the cross contamination from test pad to test pad.

Absorbent materials were successively impregnated with the following solutions and dried at 25 degrees C:

For the pH pad:

Solution 1

0.5 g Bromcresol Green Sodium Salt

0.5 g Thymol Blue Sodium Salt

Add a quantity of distilled water sufficient to make 1 L total volume of solution

Adjust the pH of the solution to 6.0 with 0.5 N NaOH or 0.5 N HCl

solutions.

For the glucose pad:

Solution 1

4.0 g glucose oxidase

1.0 % urea peroxide

5.0 mL o-tolidine

1.0 N Phosphate buffer

Add a quantity of distilled water sufficient to make 1 L total volume of solution

Adjust the pH of the solution to 7.0 with 0.5 N NaOH or 0.5 N HCl solutions.

The LFD Hybrid of this example depicts the detection both the glucose and pH concentration of a urine solution. Two paper carrier matrices (S&S, 593 grade filter paper), one impregnated with the compositions of solution 1 for glucose and the other impregnated with the compositions of solution 1 for pH were prepared. The papers were then cut into test pads 5 mm by 5 mm in size. The papers were then dried using forced air. The 5 by 5 mm dried impregnated test pads were then placed onto a 5 mm by 70 mm long strip of nitrocellulose membrane (S&S FastTrack™ NC) at one end, so that the 5 by 5 mm test pads covered the last 5 mm and last 10 to 15 mm area of the nitrocellulose membrane (i.e. next to each other 5 mm apart) at the opposite end of the site where the test sample was introduced. Both pads were separated from each other by a space of 5 mm and were in fluid contact with nitrocellulose lateral flow paper. The nitrocellulose membrane was capable of transporting a liquid by capillary action or wicking from one end of the LFD Hybrid to the other in approximately 60 seconds. Therefore, the LFD Hybrid had the dimensions of 5 mm wide by 70mm and could be backed by or in contact with strips of glass fiber filter material (e.g. S&S 30 grade) to aid in controlling the wicking or capillary action, or other solid support material could be used such as paper, plastic, or vinyl or other suitable material to back the lateral flow material and provide support if needed.

The embodiment depicted in this example had a starting point or origin at which the sample was placed at 5 mm from one end of the strip, and approximately 60 mm from the site of where the test pads were placed in fluid contact with the strip as shown in FIG. 1B. In this example the 5 mm by 5 mm impregnated test pads were placed on top of the lateral flow paper and thus both were in fluid contact with the said paper.

The measurement of the glucose and pH concentrations of fluid, in this case urine, occurred in the same manner as explained by EXAMPLES 11 and 12.

EXAMPLE 14

This example illustrates an LDF Hybrid device for urobilinogen. Absorbent material was successively impregnated with the following solutions and dried at 25 degree C:

Solution 1

4-methoxybenzene diazonium fluoborate 0.5 g

oxalic acid 9.0 g

methanol 100 mL

Dilute to final volume of 100 mL

A paper carrier matrix (S&S, 593 grade filter paper) was impregnated with the compositions of solution 1. The paper was then dried using forced air. The paper was then cut into test pads 5 mm by 5mm. The dried impregnated test pad was then placed at approximately 35 mm (in the middle) of a 5 mm wide by 70 mm long nitrocellulose membrane (S&S FastTrack NC) to make fluid contact with nitrocellulose lateral flow paper. The nitrocellulose membrane was capable of transporting a liquid by capillary action or wicking from one end of the lateral flow device to the other in approximately 60 seconds. In this example, the LFD Hybrid had the dimensions of 5 mm wide by 70mm long and could be backed by or in contact with strips of glass fiber filter material (e.g. S&S 30 grade) to aid in controlling the wicking action, or other solid support material could be used.

The starting point or origin at which the sample is placed on the test device was 5 mm from one end of the strip, and 35 mm from the site of where the test pad was placed in fluid contact with the strip. For simplicity, this example had the 5 mm by 5 mm impregnated test pad placed on top of the lateral flow paper and thus in fluid contact with said paper.

The LFD Hybrid device worked as follows. The starting point or origin at which the sample was placed on the test device was 5 mm from one end of the strip, and 35 mm from site where the chemically impregnated test pad was in fluid contact with the lateral flow paper. (The test pad could be placed on top of the lateral flow paper making fluid

contact with the lateral flow paper from the bottom side of the test pad, or the lateral flow paper could touch the paper from the side of the test pad and remain in fluid contact with the test pad. Or the lateral flow paper could rest on top of the edge of test pad or be attached and in fluid contact with the test pad in some other manner.)

A drop of urine (approximately 50 uL) was applied at the starting point or origin of the strip. The urine then migrated to the opposite or terminal end of the strip. As the urine migrated across the lateral flow material (nitrocellulose) and came into contact with the test pad (filter page paper), the urine saturated the pad and, if positive, caused a chemical reaction between the impregnated chemicals and urobilinogen in the urine. If the sample was positive, with a concentration of 0.5 mg/dL urobilinogen or more, a brick reddish color developed on the test pad indicating a positive (greater than 0.5 mg/dL urobilinogen) for the presence of positive levels of urobilinogen. This color was compared to a color chart showing the different colors from colorless (white background)) to a dark brick red depending upon the concentration of the urobilinogen.

If the sample was negative, with a concentration of less than 0.5 mg/dL of urobilinogen present, no reaction occurred and no color developed, indicating a negative result. This negative result color could then be compared to a color chart showing the different colors from no color developed (negative) to dark brick red depending upon the concentration of the urobilinogen. The reaction on the test pad was rapid thus the test results could be observed immediately.

EXAMPLE 15

This example illustrates a LFD Hybrid device for ascorbic acid. Absorbent material was successively impregnated with the following solutions and dried at 25 degrees C:

Solution 1

2,6-dichlorophenolindophenol	1.0 g
metaphosphoric acid	9.0 g

Dilute to final volume of 100 mL with distilled water.

The above solution could be replaced with Tillman's reagent.

A paper carrier matrix (S&S, 593 grade filter paper) was impregnated with the compositions of solution 1. The paper was then dried using forced air. The paper was then cut into test pads 5 mm by 5mm. The dried impregnated test pad was then placed at approximately 35 mm (in the middle) of a 5 mm wide by 70 mm long nitrocellulose membrane (S&S FastTrack NC) and made fluid contact with nitrocellulose lateral flow paper. The nitrocellulose membrane was capable of transporting a liquid by capillary action or wicking from one end of the lateral flow device to the other in approximately 60 seconds. In this example, the LFD Hybrid device had the dimensions of 5 mm wide by 70mm long and could be backed by or in contact with strips of glass fiber filter material (e.g. S&S 30 grade) to aid in controlling the wicking action, or other solid support material could be used.

The starting point or origin at which the sample is placed on the test device was 5 mm from one end of the strip, and 35 mm from the site of where the test pad was placed in fluid contact with the strip. For simplicity, this example had the 5 mm by 5 mm impregnated test pad placed on top of the lateral flow paper and thus in fluid contact with the paper.

The starting point or origin at which the sample was placed on the test device was 5 mm from one end of the strip, and 35 mm from site where the chemically impregnated test pad was in fluid contact with the lateral flow paper. The test pad was placed on top of the lateral flow paper making fluid contact with the lateral flow paper from the bottom side of the test pad, or the lateral flow paper could touch the paper from the side of the test pad and remain in fluid contact with the test pad. Or the lateral flow paper could rest on top of the edge of test pad or be attached and in fluid contact with the test pad in some other manner.

A drop of urine (approximately 50 uL) was applied at the starting point or origin of the strip. The urine then migrated to the opposite or terminal end of the strip. As the urine migrated across the lateral flow material (nitrocellulose) and came into contact with the test pad (filter page paper), the urine saturated the pad and, if positive, caused a chemical reaction between the impregnated chemicals and ascorbic acid in the urine. If the sample was positive, with a concentration of 10.0 mg/dL ascorbic acid or more, a decoloration of

a dark blue to salmon pink color developed on the test pad indicating a positive (greater than 10.0 mg/dL ascorbic acid) for the presence of positive levels of ascorbic acid. This color could then be compared to a color chart showing the different colors from dark blue (white background on plastic strip) to a salmon pink depending upon the concentration of the ascorbic acid.

If the sample was negative, with a concentration of less than 10.0 mg/dL of ascorbic acid present, no reaction occurred and no color developed (or lack or decoloration), indicating a negative result. This negative result color could then be compared to a color chart showing the different colors from no color change (negative) to salmon pink depending upon the concentration of the ascorbic acid. The reaction on the test pad was rapid thus the test results could be observed immediately.

EXAMPLE 16

This example illustrates a LFD Hybrid device for protein. Absorbent material was successively impregnated with the following solutions and dried at 25 degrees C:

Solution 1

sodium citrate	13.0 g
citric acid	5.0 g
lauroyl sarcosine	0.7 g

Add 500 ml distilled water and bring all of the above into solution then bring the solution to 1000 mL with methanol.

Solution 2

tetrabromphenol blue	1.0 g
magnesium sulfate	7.0 g

Bring solution 2 to a volume of 1000 mL with methanol.

A paper carrier matrix (S&S, 593 grade filter paper) was impregnated with the composition of solution 1. The paper was then dried using forced air. The paper carrier matrix was then impregnated with the composition of solution 2. The paper was then dried. The paper was then cut into test pads 5 mm by 5mm. The dried impregnated test pad was then placed at approximately 35 mm (in the middle) of a 5 mm wide by 70 mm long nitrocellulose membrane (S&S FastTrack NC) to make fluid contact

with nitrocellulose lateral flow paper. The nitrocellulose membrane was capable of transporting a liquid by capillary action or wicking from one end of the lateral flow device to the other in approximately 60 seconds. In this example, the LFD Hybrid device had the dimensions of 5 mm wide by 70mm long and could be backed by or in contact with strips of glass fiber filter material (e.g. S&S 30 grade) to aid in controlling the wicking action, or other solid support material could be used.

The starting point or origin at which the sample is placed on the test device was 5 mm from one end of the strip, and 35 mm from the site of where the test pad was placed in fluid contact with the strip. For simplicity, this example had the 5 mm by 5 mm impregnated test pad placed on top of the lateral flow paper and thus in fluid contact with the paper.

The starting point or origin at which the sample was placed on the test device was 5 mm from one end of the strip, and 35 mm from site where the chemically impregnated test pad was in fluid contact with the lateral flow paper. The test pad could be placed on top of the lateral flow paper making fluid contact with the lateral flow paper from the bottom side of the test pad, or the lateral flow paper could touch the paper from the side of the test pad and remain in fluid contact with the test pad. Or the lateral flow paper could rest on top of the edge of test pad or be attached and in fluid contact with the test pad in some other manner.

A drop of urine (approximately 50 uL) was applied at the starting point or origin of the strip. The urine then migrated to the opposite or terminal end of the strip. As the urine migrated across the lateral flow material (nitrocellulose) and came into contact with the test pad (filter page paper), the urine saturated the pad and, if positive, caused a chemical reaction between the impregnated chemicals and protein in the urine. If the sample is positive, with a concentration of 10.0 mg/dL protein or more, a light green to dark green color developed on the test pad indicating a positive (greater than 10.0 mg/dL protein) for the presence of positive levels of protein. This color could then be compared to a color chart showing the different colors from yellow (white to yellow background) to a dark green depending upon the concentration of the protein.

If the sample is negative, with a concentration of less than 10.0 mg/dL of protein present, no reaction occurred and no color developed (or lack or decoloration), indicating a negative result. This negative result color could then be compared to a color chart showing the different colors from yellow color (negative) to dark green depending upon the concentration of the protein. The reaction on the test pad is rapid thus the test results could be observed immediately.

EXAMPLE 17

This example illustrates a LFD Hybrid device for blood (hemoglobin). Absorbent material was successively impregnated with the following solutions and dried at 25 degrees C:

Solution 1

sodium citrate	13.0 g
ethylenediamine-tetraacetic acid sodium salt	5.0 g
dioctyl sodium sulphosuccinate	0.7 g
urea peroxide	2.3 g
phosphoric acid trimorpholide	7.0 g
ethanol	23.0 ml

Add distilled water and bring all of the above into solution then bring the solution to 100 mL with methanol. Then pH the solution to 5.25.

Solution 2

3,3',5,5'-tetramethylbenzidine	1.0 g
phenanthridine	0.3 g

Bring solution 2 to a volume of 100 mL with toluene.

A paper carrier matrix (S&S, 593 grade filter paper) was impregnated with the composition of solution 1. The paper was then dried using forced air. The paper carrier matrix was impregnated with the composition of solution 2. The paper was then dried. The paper was then cut into test pads 5 mm by 5mm. The dried impregnated test pad was then placed at approximately 35 mm (in the middle) of a 5 mm wide by 70 mm long nitrocellulose membrane (S&S FastTrack NC) and made fluid contact with nitrocellulose lateral flow paper. The nitrocellulose membrane was capable of transporting a liquid by capillary action or wicking from one end of the lateral flow device to the other in

approximately 60 seconds. In this example, the LFD Hybrid device had dimensions of 5 mm wide by 70mm long and could be backed by or in contact with strips of glass fiber filter material (e.g. S&S 30 grade) to aid in controlling the wicking action, or other solid support material could be used.

The starting point or origin at which the sample was placed on the test device was 5 mm from one end of the strip, and 35 mm from the site of where the test pad was placed in fluid contact with the strip. For simplicity, this example had the 5 mm by 5 mm impregnated test pad placed on top of the lateral flow paper and thus be fluid contact with the paper.

The starting point or origin at which the sample was placed on the test device was 5 mm from one end of the strip, and 35 mm from site where the chemically impregnated test pad was in fluid contact with the lateral flow paper. The test pad could be placed on top of the lateral flow paper making fluid contact with the lateral flow paper from the bottom side of the test pad, or the lateral flow paper could touch the paper from the side of the test pad and remain in fluid contact with the test pad. Or the lateral flow paper could rest on top of the edge of test pad or be attached and in fluid contact with the test pad in some other manner.

A drop of urine (approximately 50 uL) was applied at the starting point or origin of the strip. The urine then migrated to the opposite or terminal end of the strip. As the urine migrated across the lateral flow material (nitrocellulose) and came into contact with the test pad (filter page paper), the urine saturated the pad and, if positive, caused a chemical reaction between the impregnated chemicals and blood in the urine. If the sample was positive, with a concentration of 15.0 mcg/dL blood (hemoglobin) or more, a light green to dark green color developed on the test pad indicating a positive (greater than 15.0 mcg/dL blood) for the presence of positive levels of blood. This color was compared to a color chart showing the different colors from yellow (white to yellow background) to a dark green depending upon the concentration of the blood.

If the sample is negative, with a concentration of less than 10.0 mg/dL of blood present, no reaction occurs and no color developed (or lack or decoloration), indicating a negative result. This negative result color could then be compared to a color chart showing

the different colors from yellow color (negative) to dark green depending upon the concentration of the blood. The reaction on the test pad is rapid thus the test results can be observed immediately.

Test pads for other analytes could be added or substituted for the test pads of Example 13. Sizes of the test pads and lateral flow material in this and other examples can obviously be changed. The LFD Hybrid technology herein may be used as a stand alone POC device or be incorporated into other testing devices.

The examples presented in this application relate to test devices and methods of detecting adulterants in urine but could also work in other matrices such as blood, saliva, sweat extracts, serum, hair homogenates, gastric contents, cerebral spinal fluid or other fluids that come from the human body. The LFD Hybrid could also be used to detect specific gravity, urobilinogen, glucose, bleach, nitrite, oxidants, bilirubin, leukocyte esterase, ketone, protein, or other common urine assays.

It is understood that variations or modifications in the following embodiments may be made by someone skilled in the art without departing from the spirit and scope of the invention. All such modifications and variations are to be included within the scope of the invention as defined in the appended claims:

I claim:

1. A method for the detection of the presence or absence of glutaraldehyde in a sample of bodily fluid comprising the steps of:

(A) preparing a dry chemistry test means by impregnating a solid, carrier matrix with an indicator capable of producing a detectable response and a buffer;

(B) drying the impregnated, solid carrier matrix;

(C) contacting said dry chemistry test means with the sample of bodily fluid; and

(D) observing the detectable response in the presence or absence of glutaraldehyde.

2. The method according to claim 1 wherein the indicator is selected from the group consisting of sodium nitroprusside, ferric chloride, salicylates, phenol, antipyrine and sodium nitroprusside analogs.

3. The method according to claim 1 wherein the buffer is selected from the group consisting of phosphate, borate, borax, sodium tetraborate decahydrate, sodium perchlorate, sodium chlorate, sodium carbonate, tris base, tris acid, MES, BIS-TRIS, ADA, ACES, PIPES, MOPSO, BIS-TRIS PROPANE, BES, MOPS, TES, HEPES, DIPSO, MOBS, TAPSO, TRIZMA, HEPPSO, POPSO, TEA, EPPS, TRICINE, BLYC-GLY, BICINE, HEPBS, TAPS, AMPD, TABS, AMPSO, CHES, CAPSO, AMP, CAPS, CABS, hydrochloric acid, phosphoric acid, lactic acid, potassium hydrogen tartrate, potassium hydrogen phthalate, calcium hydroxide, bicarbonate, sodium hydroxide, potassium hydroxide, oxalate, and succinate.

4. The method of claim 1 wherein the detectable response is a color change visible to the human eye or in the visible light spectrum.

5. A method for the detection of the presence or absence of glutaraldehyde in a sample of urine comprising the steps of:

(A) preparing a dry chemistry test means by impregnating a solid, carrier matrix with an indicator selected from the group consisting of sodium nitroprusside, ferric chloride, salicylates, phenol, antipyrine and sodium

nitroprusside analogs and a buffer selected from the group consisting of phosphate, borate, borax, sodium tetraborate decahydrate, sodium perchlorate, sodium chlorate, sodium carbonate, tris base, tris acid, MES, BIS-TRIS, ADA, ACES, PIPES, MOPSO, BIS-TRIS PROPANE, BES, MOPS, TES, HEPES, DIPSO, MOBS, TAPSO, TRIZMA, HEPPSO, POPSO, TEA, EPPS, TRICINE, BLYC-GLY, BICINE, HEPBS, TAPS, AMPD, TABS, AMPSO, CHES, CAPSO, AMP, CAPS, CABS, hydrochloric acid, phosphoric acid, lactic acid, potassium hydrogen tartrate, potassium hydrogen phthalate, calcium hydroxide, bicarbonate, sodium hydroxide, potassium hydroxide, oxalate, and succinate; and

(B) drying the impregnated, solid carrier matrix;

(C) contacting said dry chemistry test means into with the sample of urine; and

(D) observing the detectable response in the form of a color change visible to the human eye or in the visible light spectrum in the presence or absence of glutaraldehyde.

6. The method of claim 1 wherein the bodily fluid is selected from the group consisting of serum, whole blood, cerebral spinal fluid, gastric fluid, hair homogenates, sweat extracts, and saliva.

7. A method for the detection of an analyte in a sample of bodily fluid comprising the steps of:

(A) preparing a dry chemistry test means by successively impregnating a solid, carrier matrix with at least one reagent capable of providing a detectable response to the presence of the analyte;

(B) drying the impregnated, solid carrier matrix;

(C) disposing the dried impregnated solid carrier matrix proximate to a lateral flow material;

(D) contacting the sample with the dry chemistry test means; and

(E) observing the detectable response.

8. The method of claim 7 comprising the additional step of laminating the impregnated solid carrier matrix onto a lateral flow membrane.

9. The method according to claim 7 wherein the detectable response is a color change visible to the human eye or in the visible light spectrum.

10. The method according to claim 7 wherein the sample of bodily fluid is selected from the group consisting of urine, serum, whole blood, cerebral spinal fluid. gastric fluid, hair homogenates, sweat extracts, and saliva.

11. A device for the detection of the presence or absence of an analyte in a sample of bodily fluid comprising:

(A)lateral flow means for transporting a portion of sample throughout said means;

(B test pad means being positioned in fluid contact with said lateral flow means;

(C) test pad means comprising a reagent capable of producing a detectable response in the presence of an analyte.

12. The device of claim 11 wherein the analyte is selected from the group consisting of: pH, specific gravity, glucose, urobilinogen, bleach, nitrite, oxidants, bilirubin, ketone, leukocyte esterase, chromate, glutaraldehyde, creatinine, blood (hemoglobin), ascorbic acid, protein, calcium and any other chemistry analyte.

13. The device of claim 11 wherein the sample of bodily fluid is selected from the group consisting of urine, serum, whole blood, cerebral spinal fluid, gastric fluid, hair homogenates, sweat extracts, and saliva.

14. The method according to claim 11 wherein the detectable response is a color change visible to the human eye or in the visible light spectrum.

FIG. 1

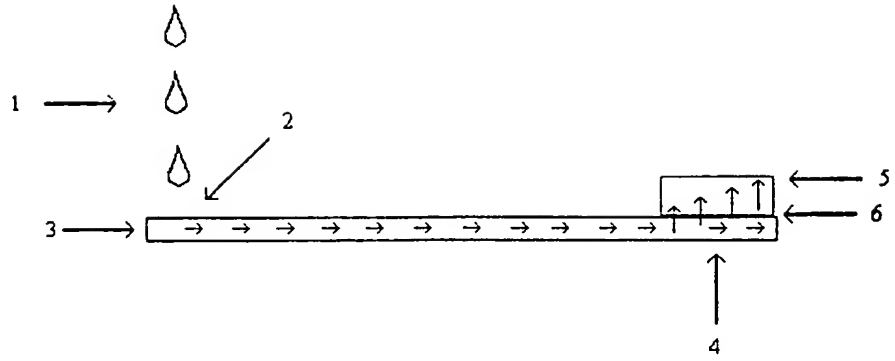

FIG. 1A

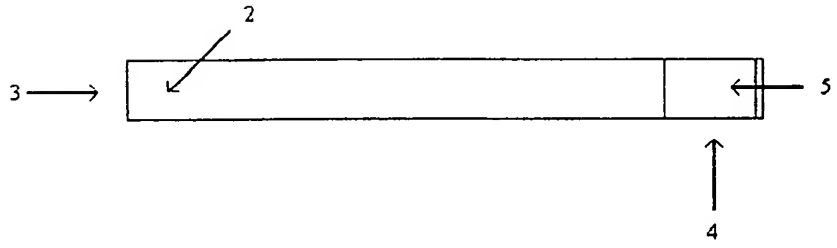

FIG. 1B

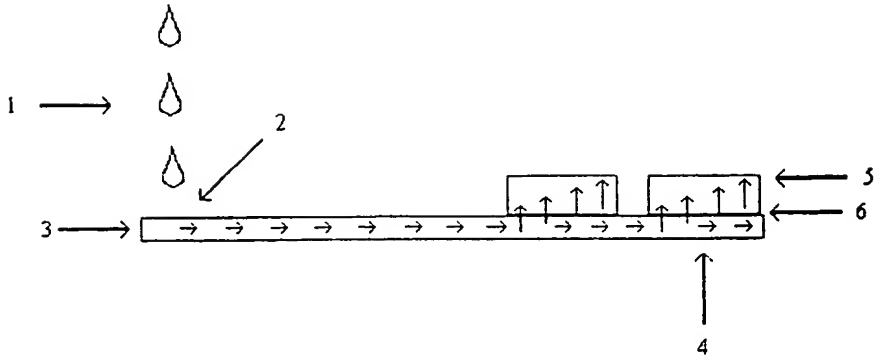

FIG. 2

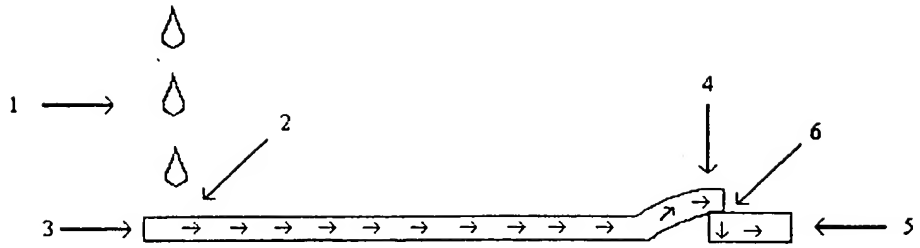

FIG. 3